Journal of Applied Botany and Food Quality 93, 217 - 224 (2020), DOI:10.5073/JABFQ.2020.093.026

[1]Department of Horticulture, Agrifood Research and Technology Centre of Aragon (CITA), Zaragoza, Spain
[2]AgriFood Institute of Aragon – IA2 (CITA-University of Zaragoza), Zaragoza, Spain

Sugar content and organic acid profiles of local apple cultivars recovered from mountain zones

Lourdes Castel[1], Ana Pina[1,2], Patricia Irisarri[1], Pilar Errea[1,2]*

(Submitted: April 6, 2020; Accepted: October 2, 2020)

Summary

Ancient apple cultivars grown in local areas have so far been largely unexplored and may attract a large share of consumers oriented towards natural foods evoking ancient flavors. In this work, 34 traditional apple cultivars of the Pyrenees were analyzed in terms of sugar and acidity profile and their relation, as alternative fruits for a growing demand in society regarding quality and nutritional properties. The results show a wide range between cultivars of analyzed variables, especially in terms of acid, pH and soluble solids, with high standard deviation values. The results were higher than in other similar studies of commercial apple cultivars, as well as higher values related to other local accessions. The large differences between cultivars can be attributed to the origin of the plant material, since all cultivars were grown under the same geographical conditions and with the same applied agronomic practices. The associations found in this study provide information about the nutritional content of the analyzed apples and their organoleptic and physicochemical qualities, and could be useful in targeting specific consumer requirements. In conclusion, this study highlights the suitability of these local accessions recovered in the Pyrenees as key genetic resources to be used in future breeding programs, as well as its potential use for different products which would open the door to new varieties and new consumption alternatives.

Key words: apple cultivars, ancient varieties, sugars, organic acids, PCA

Introduction

The genetic diversity of apple cultivars represents an unique and invaluable natural resource (VANDERZANDE et al., 2017). In mountainous areas from Aragon (north-eastern Spain), generations of farmers performing selection processes in their orchards and home gardens have given rise to a wide diversity of quality fruit-plant material that constitutes an extraordinary genetic heritage of germplasm. In the past, apple production was based on local varieties, but the great varietal reconversion in the 1960s led to the abandonment of many of these varieties that had an important significance in local food. Currently, the world production of about 70 million metric tons/year is based on only a small number of cultivars: Only seven apple cultivars account for more than 50% of the production in Europe (VAN NOCKER et al., 2012). The massive use of few related commercial cultivars has dramatically reduced the genetic diversity of local apple varieties, and many interesting and well-adapted traditional and local varieties, which are known to contain many desirable attributes, have been lost. The reduction in genetic variability is an important limitation of the ability to respond to new needs and challenges. The selection programmes for new apple varieties have resulted in a decrease in the diversity of apple genetic background (CORNILLE et al., 2012). But the development of new apple cultivars with desirable characteristics and a wide genetic base is dependent upon breeders having access to as much genetic diversity as possible. And now, there is a growing demand in society regarding quality and health, which is increasing the interest in varieties encoding for fruit quality and disease resistance traits that offer new opportunities for cultivation and use as raw material for breeding.

This local germplasm is cultivated mainly in marginal areas, is well adapted to the local environment and is resistant to biotic and abiotic stress. The study of the species and fruit varieties that have survived this abandonment, their potential in relation to other more widespread species and the understanding of the physiological characteristics that make them worthy of this survival constitute a fundamental aspect, not only of the preservation of this richness and diversity genetics, but also of the value of the potential development of these species in these areas of possible fruit regeneration. Local apple accessions from these mountain zones were collected and recovered and their genetic identity and variability analysed (PINA et al., 2014). An important range of genetic variability was detected, suggesting interesting differences in the physical and chemical composition and nutritional properties of the fruit, which could increase the value of these local accessions.

The chemical composition of apples is very complex. Among the most important constituents are sugars and organic acids (LANZERSTORFER et al., 2014). Their contents and especially their ratio greatly affect the desirability of apple for direct consumption and their suitability for processing (PETKOVSEK et al., 2007). The sugar profile of the fruit pulp has an effect on the organoleptic properties and nutritional value of these fruits. The most significant sugars are fructose, glucose, sucrose and sorbitol (ZHANG et al., 2010; LARSEN et al., 2019). The organic acids in apples include malic, citric, shikimic, fumaric, and quinic acids (MAPSON, 1970).These make an important contribution to the flavor of the fruit and also to their stability, colour, nutrition, acceptability and keeping qualities. The balance between sweetness and acidity is also responsible for the taste and flavor of apples (PERSIC et al., 2017; PETKOVSEK et al., 2007; HECKE et al., 2006).

Several studies have addressed the chemical composition of different apple cultivars, but most have focused on a limited number of cultivars from among those most widely consumed and appreciated (KEENAN et al., 2012; GUAN et al., 2015). As a result, very few data are available in the literature on the nutritional properties of geographically limited area, particularly traditional apple cultivars (JAKOBEK and BARRON, 2016; LARSEN et al., 2017). Such a lack of information prevents the use of these germplasms for the genetic improvement of new cultivars and re-evaluating local food products, something which may attract a large number of consumers looking for natural foods that evoke ancient flavors.

To the best of our knowledge, the sugar and organic acid compositions of local apples recovered from the mountainous areas of the Pyrenees have not been investigated until now. The main objectives of this paper were to characterize local apple cultivars from mountainous areas by analyzing their sugar and acid contents, total soluble solids (TSS, °Brix) and pH, as well as the study of the relationships established between the various characteristics in order to increase

* Corresponding author

the value of this unique local material, especially as far as its chemical composition is concerned.

Material and Methods

Plant material

Thirty-four apple accessions (*Malus domestica* Borkh) and one reference cultivar 'Golden Delicious' were analyzed. These accessions belong to the CITA germplasm collection and were recovered on various prospecting missions to mountainous areas of Aragon (north-eastern Spain) since 2001. They were collected in the Pyrenean localities in Aragon and included samples from old, abandoned trees and small farms and were shown to be unique genotypes by SSR markers (Pina et al., 2014).These trees were propagated vegetatively and were established in collections located in Bescós de la Garcipollera (Huesca, Aragon) at an altitude of 900 m.

Apple fruits were collected between August and October at the ripening period of each cultivar. Fifteen fruits were selected from each accession for analysis.

Morphological data

Harvest time, fruit weight and firmness of flesh were recorded for each cultivar according to Upov (1974), IBPGR (Watkins and Smith, 1983) and Royo Diaz et al. (2017) guidelines.

Fruits were hand-picked at technological maturity by a single person to ensure consistency of the maturity grade. The harvest date decision was based on development in fruit removal force, sensory evaluation (color, softness, smell, and taste) and supported by the determination of the starch-iodine test CTIFL scale (Platon, 1995) reached at least an index of 6. Firmness was measured using a Digital fruit firmness teste (Model TR Turoni) with a 11 mm plunger tip.

'Golden Delicious' was used as a reference cultivar, which matured in the first two weeks of October. In this way, five different groups were classified from the collection dates (1 – very early, 3 – early, 5 – medium, 7 – late or very late)

Determination of total soluble solids, acid content and pH

Soluble solid content (SSC) were determined from the juice of the samples analysed in each batch. The juice was obtained by liquefying apple pieces with skin and pulp of at least 10 fruits. Juice was filtered through a paper funnel (filter paper circles-folded, ø 185 mm, Chmlab group, Barcelona, Spain), to separate the solid phase from the liquid one. Once the juice was obtained, the SSC were measured at 20 °C using a refractometer (Pocket Refractometer Pal-1, Atago, Tokyo, Japan) and expressed as °Brix.

The acid content and pH were determined at 20 °C, using a digital pH meter calibrated with pH 4 and pH 7 buffers measured with a model760 Basic Titrino (785 DMP Titrino, Metrohm, Herisau, Switzerland). In addition, 25 mL of the filtered juice was placed in a beaker. The sample was taken to the valorimeter (760-Sample Change, Metrohm, Herisau, Switzerland) and the juice was titrated with 0.1 N NaOH. The results obtained were: pH and total acid (TA), and malic and citric acid contents.

Determination of sugars (sucrose, glucose, fructose and xylose)

Individual sugar contents (sucrose, glucose, fructose and xylose) were determined according to the method described by Blanco et al. (1988) using high-performance liquid chromatography (HPLC - ICP Activa Horiva Jobin Yvon, Kyoto, Japan). Juice (1 mL) was weighed and diluted with Milli-Q water to 25 mL. The samples were purified and filtered through a methanol and polyvinylidene difluoride-activated Sep-Pack cartridge (Waters Cromatography, Cerdanyola del Vallés, Spain) with a 0.45 µm filter. Then, 20 µl of the treated sample was injected into the chromatographic system.

The separation was performed using a Waters SUGAR-PAK I (Waters Cromatography, Cerdanyola del Vallés, Spain) (300 × 6.0 mm) 10 µm column, at 90 °C, using as a mobile phase a solution of 50 mg/L Na_2Ca EDTA at a flow rate of 0.44 mL/min.

Statistical analysis

All traits were measured, or scored, for each genotype separately over the two-year period, and the two-year means were calculated. All the statistical analyses were performed using SPSS Statistics version 21.0 (IBM, New York, U.S.A). The minimum and maximum values, standard deviation, and variance were calculated for each accession studied. The normality of the data was analysed using the Shapiro-Wilk test, and traits showing normal distribution were analysed by one-way analysis of variance (ANOVA), considering genotype and year as independent factors. Traits that did not meet the normality criteria were analysed with the Kruskal-Wallis nonparametric test. In addition, correlations between and within traits for each year were analysed using Pearson coefficients. Correlations were calculated using the raw data from the two-year period, according to Pearson's test at $p \leq 0.01$ and $p \leq 0.05$.

The results for the individual sugars were compared using one-way ANOVA and the differences determined tested with the Kruskal-Wallis test at a significance level of 0.05. Data for each genotype were averaged and mean values used as estimated genotypic values. Multidimensional scaling (PROXSCAL algorithm) analysis was applied to the studied accessions as an attempt to identify how the studied components are distributed based on their nutritional compound content and organoleptic quality. Applying multidimensional scaling (MDS) generated a spatial map that facilitated an intuitive understanding of the relationships between the objects represented on the map. The distances between the objects indicate their (dis) similarities: similar objects are represented by closely spaced points and dissimilar objects by points that are further apart. The dimensions are factors with real meaning that make it possible to explain the differences between groups.

The component matrix was evaluated and orthogonal factors rotated using variance maximising (Varimax). Finally, a dendrogram was constructed to evaluate the homogeneity of the clusters using the inter-group linkage method.

Results and discussion

Harvest time and morphological characterization in the local apple accessions

Harvest times showed five different groups using 'Golden Delicious' as reference cultivar: very early, early, medium, late and very late (Tab. 1). The time varied from 21 August for the earliest cultivar (accession 29) to 15 November for the latest cultivars (accessions 5, 7, 18 and 21). Most of the local accessions matured in the early and medium harvest periods.

In Spain, 87% of apple production is limited to four cultivars: 'Golden Delicious' (more than 50% of production), 'Gala', 'Red Delicious' and 'Fuji' (Iglesias et al., 2016), which are harvested in this region between mid-August and mid-October. Local varieties were severely displaced by more modern ones from other countries, and this limited variability also in traits of interest, included a wide range of collection times found in traditional cultivars. Other morphological observations (Tab. 1) showed different ranges of weight and firmness. Total weight showed values between 48.84 and 276.18 g. Cultivars producing smaller apples were accession 16. The firmness varied between 5.40 in accession 30 to 9.88 kg/cm^2 in accession 7, showing great variability as well as other studies related to local germplasm (Ramos-Cabrer et al., 2007).

Tab. 1: Different ranges of harvest time, fruit weight (gr), firmness (kg/cm²), ground colour, relative area of over colour, intensity of over colour and general shape of the 34 cultivars used in this study.

Descriptors UPOV	U_56	U_24	U_52	U_35	U_36	U_37	U_38	U_28
Cultivar	**Harvest time**	**Fruit weight (g)**	**Firmness (kg/cm²)**	**Ground colour**	**Relative area of over colour**	**Hue of over colour**	**Intensity of over colour**	**General shape**
1	Very late	93.51	8.20	Whitish green	Not visible	-	Not visible	Oblong intermediate-conical
2	Early	212.82	8.15	Yellow-green	Not visible	Red	Not visible	Short-globose conical
3	Early	158.53	6.95	Yellow-green	Very large	Orange	Medium	Flat-globose
4	Late	248.71	9.13	Whitish yellow	Two colours	-	Medium	Flat-globose
5	Very late	129.19	9.55	Not visible	Not visible	Pink	Not visible	Flat-globose
6	Very early	165.71	7.53	Whitish yellow	Two colours	Red	Light	Flat-globose
7	Late	248.75	9.88	Yellow-green	Two colours	Orange	Dark	oblong-conical
8	Medium	178.27	7.28	Yellow-green	Two colours	-	Light	Short-globose conical
9	Medium	194.37	8.95	Yellow-green	Not visible	-	Not visible	Flat-globulose
10	Early	276.18	7.93	Whitish yellow	Not visible	Orange	Not visible	Flat
11	Late	224.99	8.75	Yellow-green	Two colours	-	Light	Short-globose conical
12	Very early	181.77	9.30	Yellow-green	Not visible	Red	Not visible	Flat
13	Early	155.18	7.50	Yellow-green	Very small	-	Medium	Globose
14	Early	221.07	8.25	Green	Not visible	Reddish brown	Not visible	Globose intermediate-conical
15	Early	86.52	7.80	Green	Two colours	Reddish brown	Light	Oblong-conical
16	Very late	48.84	9.13	Yellow-green	Very small	Red	Dark	Globose intermediate-conical
17	Medium	244.05	6.48	Yellow-green	Very large	Pink	Dark	Flat-globulose
18	Medium	151.06	6.20	Yellow	Very small	-	Light	Short-globose conical
19	Late	66.45	7.58	Yellow-green	Not visible	-	Not visible	Flat
20	Early	80.79	6.60	Yellow	Not visible	Red	Not visible	Flat cronical-trunk
21	Early	247.19	8.13	Yellow	Very large	Red	Medium	Conical
22	Medium	240.90	6.90	Yellow	Very large	-	Dark	Flat Cronical-trunk
23	Early	213.86	7.35	Green	Not visible	Red	Not visible	Flat Cronical-trunk
24	Early	265.33	7.45	Yellow-green	Very large	Red	Dark	Flat Cronical-trunk
25	Early	199.47	9.63	Not visible	Very large	Pink	Dark	Globose intermediate-conical
26	Very early	260.00	8.10	Yellow-green	Very small	-	Light	Flat-globose
27	Early	167.11	6.08	Yellow	Not visible	-	Not visible	Oval Cronical-trunk
28	Very early	123.67	6.60	Whitish yellow	Not visible	Red	Not visible	Flat-globose
29	Very early	182.43	7.23	Whitish yellow	Very large	-	Dark	Short-globose conical
30	Early	155.05	5.40	Whitish yellow	Not visible	Pink	Not visible	Flat-globose
31	Early	133.75	6.85	Yellow	Very small	Pink	Dark	Short-globose conical
32	Very early	147.10	6.25	Yellow	Very large	Orange	Dark	Flat-globose
33	Medium	198.78	7.75	Green	Very small	Orange	Light	Short-globose conical
34	Medium	162.64	8.45	Yellow-green	Two colours		Medium	Flat-globose

pH, total acid (TA), malic, citric and soluble solid content

Tab. 2 shows the analytical results for pH, acid composition and SSC (°Brix) for the 34 local apple accessions. A great variability was found in terms of pH, TA and SSC. TA contents varied from 0.334 g/kg in accession 10 to 12.055 g/kg in accession 18. These data are higher than those recorded in other research carried out on Granny Smith and other local apple cultivars (Begic-Akagi et al., 2014), which showed values between 0.62 and 15.36 g/kg, and those reported by Hecke et al. (2006), who reported TA values of commercial cultivars between 6.26 and 14.12 g/kg. Some studies have determined that TA had a negative correlation with sensory acceptability indicating it had a negative impact on panellist acceptability (Keenan et al., 2012). In general, many consumers determine apple quality based on their balance of sweetness and acidity, and good balance between acidity and sweetness should be a good criterion in selecting apples for processing applications.

The main acids involved in the acidity of the fruit are organic. These compounds, along with the sugars, influence the organoleptic perception of sweetness and the aroma of the apple (Harker et al., 2006; Aprea et al., 2017).

The content of organic acids in fruit juices not only influences their flavour but also their stability, nutrition, acceptability and keeping quality. Besides their importance in flavour, acids are important in gelling product processing because they affect the gelling properties of pectin. Also, the non-volatile malic, citric and quinic acids mediate the sour-to-astringent taste of apples (Chapman and Horvat,

Tab. 2: Descriptive statistics for the variables of TA (total acid), Malic acid, Citric acid pH, and SSC (Soluble Solids Content ° Brix) of the 34 local accessions studied. For each trait, minimum, maximum and mean values and standard deviation (SD) are presented. %CV percent coefficient of variation

	Min	Max	Mean	SD	%CV
% TA	0.33	12.06	1.72	2.32	1.35
Malic acid (g/l)	1.87	60.27	8.97	11.57	1.29
Citric acid (g/l)	1.79	57.57	8.57	11.06	1.29
pH	2.97	8.19	4.19	1.20	0.29
SSC (°Brix)	10.5	19.6	14.83	1.84	0.12

1989). The regular consumption of fruit acids is helpful in preventing illness and mild metabolic disorders in the human body. For example, fruit acids work as 'natural' tooth cleaners.

The SSC expressed as °Brix is commonly used as an estimate of fruit sweetness and is included in assessments of the postharvest quality of apples (Guan et al., 2015). In the set of accessions studied, SSC ranged between 10.5 and 19.60 °Brix (Tab. 2). These results were higher than in other similar studies of commercial apple cultivars, which recorded values of 9.5 to 15.8 (Aprea et al., 2017), 11.8 to 14 (Vieira et al., 2009) 10.48 to 14.68 (Wu et al., 2007), or 11.4 to 16.1 °Brix (Thompson-Witrick et al., 2014), as well as higher values related to other local accessions from southern Italy, with values of between 11.1 and 16.1 °Brix (Panzella et al., 2013) or non-commercial old varieties of apples cultivated in Upper Austria, of between 8 and 18.9 °Brix (Lanzerstorfer et al., 2014).

The pH values of the apple cultivars were also very variable, from 2.97 to 8.19. This range of values is higher than others reported previously. In an apple germplasm collection from Southern Brazil (Vieira et al., 2009), the pH of the apple cultivars varied from 3.90 ('Imperatriz') to 4.27 ('Fred Hough'), and for apple cultivars grown in China (Wu et al., 2007) the pH values varied between 3.59 and 4.16. In other studies carried out on commercial apple varieties, it was observed that the highest pH was between 3.46 and 4.21, and that these values were inversely correlated with the TA values (Keenan et al., 2012).

In general, there was wide-ranging variation among the apple cultivars in terms of TA content, pH and SSC, as well as harvesting time (Tab. 1, 2) with high standard deviation values. These parameters also varied greatly in the aforementioned studies and may be due to the genotype, the specific geography of the different areas, or the various agronomic practices used. Since all the local apple cultivars used in this study were grown in the same location using similar horticultural practices, the variation in physical and chemical characteristics can be attributed to genetic variability, which was previously analysed for these genotypes (Pina et al., 2014). The striking compound variability found in these local accessions provides information on the strong added value they represent.

Sugar content: sucrose, glucose, fructose, xylose, total sugars, sucrose between glucose and glucose between fructose.

Another characteristic related to fruit quality is sugar content. Tab. 3 displays the sugar distribution range for these 34 local accessions. In general, the total population of local accessions presents a wide range of values in the variables such as the ratio glucose/fructose, total sugars, sucrose and glucose. The total sugar content of the apple cultivars in the study ranged between 79.8 and 253.6 g/L with an average concentration of 150.8 g/L. This agrees with the values reported by Begic-Akagic et al. (2014) for traditional cultivars from Bosnia-Herzegovina, with values of between 80.06 and 119.7 g kg^{-1}. For 62 heritage apple cultivars, Wang (2010) reported total sugar content ranging between 92.2 and 164.9 g/L. For commercial cultivars, these values were found to range between 115 and 183 g/kg (Hecke, 2006), but also in another study on commercial cultivars (Vieira, 2009), the total sugar content (100 g/L) ranged from 11.54 ('Imperatriz') to 14.78 ('Fuji Suprema').

Tab. 3: Descriptive statistics for the variables of sucrose, glucose, xylose, fructose, total sugars, sucrose between glucose and glucose between fructose of the 34 local accessions studied. For each trait, minimum, maximum and mean values and standard deviation (SD) are presented.

	Min	Max	Mean	SD
Sucrose (g/100 mL)	0.27	8.13	3.48	2.02
Glucose (g/100 mL)	0.96	7.74	3.07	1.51
Xylose (g/100 mL)	0.00	1.26	0.38	0.28
Fructose (g/100 mL)	4.68	14.70	8.15	2.34
Total sugars (g/100 mL)	7.98	25.36	15.08	4.49
Sucrose/Glucose	0.09	4.54	1.33	0.98
Glucose/Fructose	0.13	0.85	0.38	0.14

* Total sugars: sum of the values of glucose, xylose and fructose.

The sugar composition showed a proportion similar to other related studies. The amount of sucrose, fructose and glucose ranged from 0.27 g/100 mL to 8.13 g/100 mL, from 4.68 g/100 mL to 14.7 g/100 mL and from 0.96 g/100 mL to 7.74 g/100 mL, respectively. Xylose, a major component of xyloglucans, represented only a small fraction of the measured sugars, ranging between nondetectable and 1.26 g/100 mL. Similar levels have been reported in previous investigations (Zhang et al., 2010; Celik et al., 2018). As expected, the levels of sugar composition were fructose > glucose > sucrose, similar to other works. An investigation of pomaces from 26 cultivars observed proportions of 18-31% fructose, 3.4-24% sucrose, and 2.5-12.4% glucose per absolute weight of dry apple pomace (Queji et al., 2010). Another study of 11 cultivars measured average contents of 12.7% glucose, 17.9% fructose, and 7.0% sucrose (Sato et al., 2010). These results correspond with other reports analysing commercial cultivars, which showed average levels of 53.9% fructose, 33.8% glucose and 24% sucrose (average of eight commercial cultivars) (Wu et al., 2007) or 52.39 and 33.41 g/kg for fructose and glucose (Begic-Akagi et al., 2014). However, other results, also from commercial cultivars, the analysis of sugar composition showed sucrose (41.8%) and fructose (39.1%) as the most abundant sugars, with few exceptions, followed by glucose (18.3%) (Aprea et al., 2017). The majority of cultivars studied contained more fructose and less glucose, a fact that is an advantage for diabetes patients, since it helps to keep the blood sugar level constant (Begic-Akagi et al., 2014).

The sugar content of apples differs depending on the weather conditions, cultivar, culturing technology, position and exposure of the fruits in the crown. Some local accessions with low levels of fructose could be of particular interest to consumers. Several studies have examined total fruit sugar and the increasing levels of fructose, glucose and sucrose at advanced stages of fruit maturity (Łata, 2008; Veberic et al., 2005). It is widely known that the sugar profile of pulp flesh is an important component of chemical composition and provides valuable information on the authenticity of fruit juices related to juice production and the organoleptic properties and nutritional value of apples (Waldbauer et al., 2017). It also affects the organoleptic properties and nutritional value of fruit products, although other aspects of the chemical composition should be considered when evaluating the quality of apples. Even though the sugar and organic acid contents can be influenced by environmental factors and horticultural practices in an orchard (Colaric et al., 2007), they depend mainly on plant genotype (Persic et al., 2017). Regarding en-

vironmental conditions, it has been reported that fruit characteristics of different cultivars cultivated in mountain areas are characterised by higher sugar content than those of the same cultivar cultivated in flood plain areas (IGLESIAS, 2012). These differences might be attributed to the fact that they are better adapted to conditions of increased radiation and the influence of altitude, which may affect the hormonal balance as well as the nature and quantity of compound synthesis. Pearson correlation coefficients for the different linked fruit quality traits evaluated are shown in Tab. 4 at $p < 0.01$ and $p < 0.05$. TA is associated with fruit maturity and should be evaluated according to the relationships between the soluble solid and total organic acid contents. The local accessions studied did not show a significant correlation at the 0.01 positive level ($p < 0.01$) between TA and malic and citric acid contents. Therefore, fruits with higher TA do not display more citric and malic acid. In addition, pH and SSC showed a significant correlation at the 0.05 positive levels ($p < 0.05$) with a value of the Pearson correlation coefficient r of 0.389.
Related to sugars, fructose was positively correlated with glucose ($r = 0.701$), and we also found a positive correlation between sucrose, glucose and fructose content and total sugar content ($r = 0.615$; $r = 0.775$ and $r = 0.862$, respectively). Although the higher content of fructose with respect to the other sugars was in agreement with most studies on apple cultivars, the positive correlation between glucose and total sugars, as well as negative correlations was not found in other studies (LARSEN et al., 2019).

Effect of fruit maturity classification on sugar content and SSC

To evaluate the effect of maturity stage on sugar content and SSC, the Kruskal-Wallis nonparametric test was used to compare the mean values. Simple linear and logarithmic correlation analysis was used to indicate a measure of the correlation and the strength of the relationship between these variables. Discriminant analysis (DA) is based on the extraction of linear discriminant functions of the independent variables by means of a qualitative dependent variable and several quantitative independent variables. Two processes were applied in DA: (1) stepwise DA, which selected the quantitative variables that enhance discrimination of the groups established by the dependent variable; and (2) introduction of all independent variables. The objective of this process was to avoid information loss, although the system obtained was more complex. In our results, there was a significant relationship between sucrose and SSC and between fructose and glucose. They did not show a significant relationship between the stage of maturity of the fruit and the variables of fructose and °Brix, and other variables studied.
For fructose and SSC, whereas the local accessions with an early maturity stage present higher contents of fructose and lower SSC, those with a later ripeness stage have a lower fructose content and higher SSC. However, no significant differences in the ripeness stage for these two variables were found. As well, although the distribution indicates that the early ripening accessions have different sugar contents, no significant differences were observed in the distribution of glucose and sucrose related to the ripeness stage ($p > 0.005$).

Analysis of harvest time of the collection studied related to the rest of the variables studied.

The local accessions have been clustered in five groups according to the time of harvest (Fig. 1). The dendrograms derived from hierarchical cluster analysis reveal five figures, each consisting of different number of accessions (Fig. 1). These results indicated that the local apples studied have a wide range of harvest times, characteristic of the ripening of apples in mountain areas and also linked to the biodiversity of genotypes present in the collection of local accessions.
Fig. 1a shows how to group the accessions harvested very early, giving rise to three different groups, accession 26 being the most differentiated of the group, which stands out for having content of two colours and the lowest total sugar values of the whole studied collection. The second group is formed by accession 29, noted for being very attractive to the consumer, since its epidermis is dark red with high wax content. One of the characteristics of this accession is that it is the first to be harvested, more than 15 days different from the rest of the group. The first group is characterized by having low values of total sugars, with accession 32 being the lowest.
Fig. 1b shows the dendrogram that groups the accessions of 'early' collection time into four groups. In Group I, accession 13 stands out

Tab. 4: Pearson's correlation coefficients (r) between fruit weight, firmness, harvest time, and physico-chemical parameters of the 34 local accessions studied. Correlation with a $p < 0.05$ are marked in bold.

	Fruit Weight	Firm-ness	Harvest time	pH	Malic Acid	Citric Acid	SSC	Sucrose	Glucose	Fructose	Xylose	Total Sugars	Sucrose/ Glucose	Glucose/ Fructose
%TA	**0.353***	-0.119	0.022	-0.093	-0.069	-0.069	-0.148	0.059	-0.185	-0.148	-0.108	-0.120	0.151	-0.150
Fruit Weight	1	0.173	-0.156	-0.253	0.010	0.010	-0.250	-0.150	-0.195	-0.132	0.074	-0.197	0.091	-0.209
Firmness		1	**0.421***	0.018	-0.270	-0.270	-0.134	0.231	0.123	-0.021	0.066	0.138	0.076	0.173
Harvest time			1	0.171	-0.187	-0.187	-0.085	0.241	0.129	-0.079	-0.253	0.095	0.018	0.234
pH				1	-0.089	-0.089	**0.389***	0.**374***	0.338	0.334	-0.082	0.**451****	0.001	0.121
Malic Acid					1	**1,000****	-0.058	0.049	-0.165	-0.142	-0.137	-0.116	0.129	-0.119
Citric Acid						1	-0.058	0.049	-0.165	-0.142	-0.138	-0.116	0.129	-0.119
SSC							1	**0.488****	0.038	0.303	0.284	0.**408***	0.325	-0.137
Sucrose								1	0.172	0.198	0.083	**0.615****	**0.657****	0.115
Glucose									1	**0.701****	-0.055	**0.775****	-0.426*	**0.686****
Fructose										1	0.263	**0.862****	-0.208	0.010
Xylose											1	0.218	0.217	-0.334
Total Sugars												1	0.057	0.267
Sucrose/Glucose													1	-0.399*
Glucose/Fructose														1

** The correlation is significant at the 0.01 level (bilateral).
* The correlation is significant at the 0.05 level (bilateral).

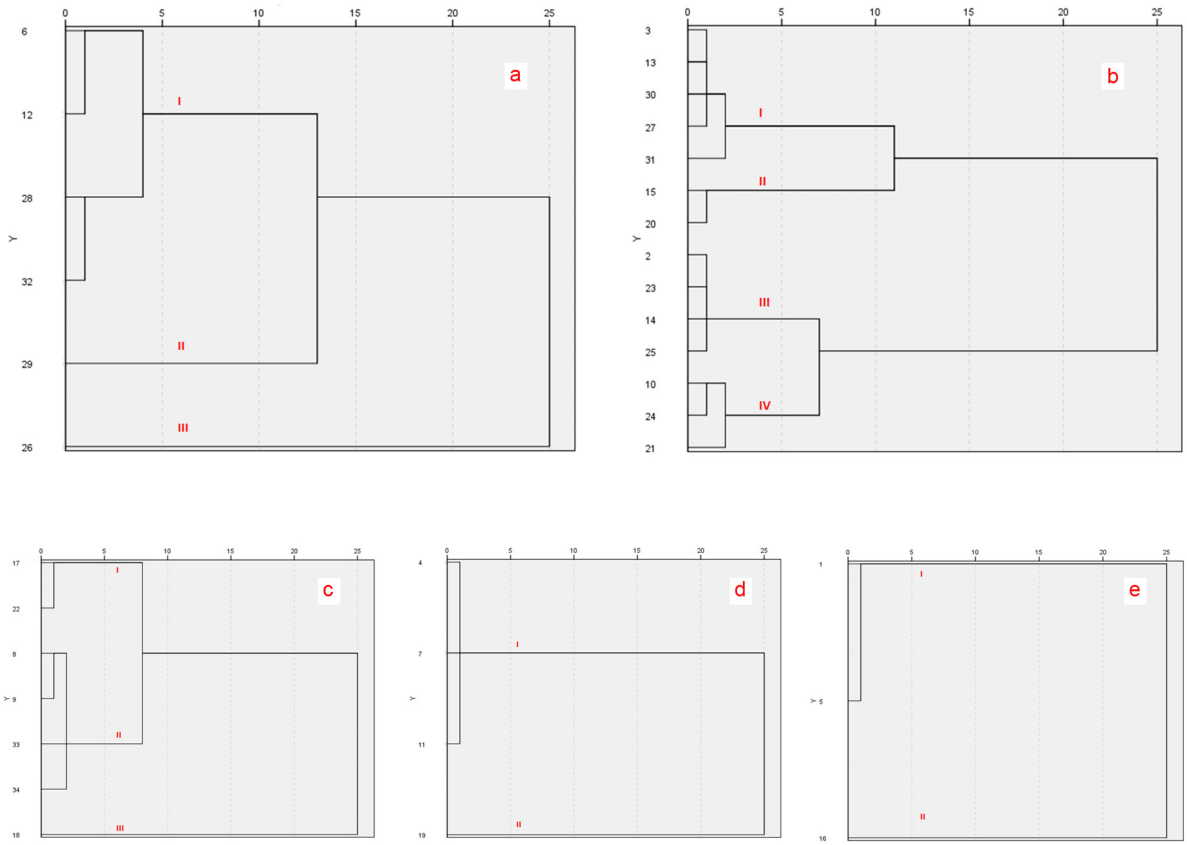

Fig. 1: Dendograms that group the variables studied according to harvesting time. Dendograms using Average Linkage (between groups) - Rescaled Distance Cluster Combine. a) Very Early b) Early c) Medium d) Late e) very late

for being the one with the lowest values of xylose, sucrose and relationship between sucrose and glucose, and accession 30 for having the highest value of SSC (19.6 °Brix) and the lowest value of firmness. Group III consists of accession 23, which has the lowest value of SSC (10.5 °Brix) of the entire collection. Within this group is accession 25, which is the one with the highest value of xylose. Group IV consists of three accessions, 10, 24 and 21. They stand out for having very extreme values: the highest in glucose and fructose in accession 21 and the highest values in weight and lowest in TA in accession 10. This accession group is very interesting for a market entry towards consumers who like sweet and low acid apples.

Fig. 1c shows the accession harvested at ‘medium’ time. That includes 7 accessions, with ranges of SSC, sugars and acids in the middle of the values. The accessions collected ‘late’ (1d), correspond to local varieties that have the highest values of total sugars and lower but above average levels of malic and citric acids: specifically, accession 16 in Group II. This does not mean that they are unpleasant in the face of consumer taste, since the very high levels of sugars and very low levels of organoleptic acidity complement each other. The smallest group (3 accessions) is harvested ‘very late’ (1e).

Multidimensional scaling analysis

Principal Component Analysis (PCA) was performed to provide easy visualisation of the complete data set in a reduced dimension plot. PCA reduces the number of variables and improves the visualisation of the data structure. Using PCA allowed us to visualise technological groups of the varieties based on their acidity and sugar profiles and identify their potential use for manufacturing processed apple products. The first two principal components (PCs) accounted for 51% of the total variance, with 32% explained by PC1 and 19% by PC2 (Fig. 2).

The representation of the relationships between the variables analysed when considering Dimension 1 (nutritional content) and Dimension 2 (organoleptic qualities that influence consumer taste and the transformation processes for elaborating transformed products) allowed the studied variables to be clearly differentiated into five groups.

If Dimension 1 (PC1) is the nutritional content, the distribution obtained suggests that genotype is the main factor determining the variability of the sugar compounds in apples and may serve in the future to select apple genotypes with a better nutritional quality or those that are most suitable for the processing industry, as has been seen in other research studies (Vieira et al., 2009).

The grouping of variables in Dimension 2 (PC2) includes all the organoleptic qualities that influence consumer taste and the transformation processes for elaborating transformed products, such as the acidity; this, and the sugar/acid ratio are responsible for the taste and flavour of apples and affect the organoleptic properties and nutritional value of fruit products (Wu et al., 2007). The results obtained in this work show that local accessions have lower sucrose content and a lower sucrose-glucose relationship than reported in other similar works. This could have an impact on the acceptance of these measures, be recommendable for consumers who are intolerant to various sugar compounds, and be useful for making mixtures in processing industries. With regard to SSC, xylose and pH, it was observed that SSC has a proportional relationship with the various types of acidity, grouped differently in the two dimensions. When the acidity increases, the SSC decreases. The acidity together with the sweetness, are the main characteristics responsible for the taste of the fruits and their acceptance by consumers.

At present, one of the objectives of the apple breeding programmes is

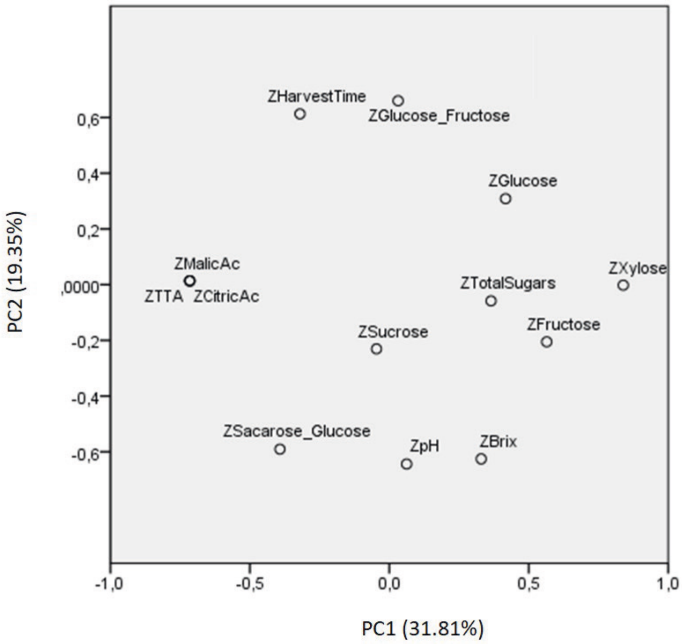

Fig. 2: Principal component analysis (PCA) of agronomical and biochemical traits of local accessions. PC 1 (nutritional content) and PC 2 (organoleptic qualities that influence consumer taste and the transformation processes for elaborating transformed products).

to improve the marketing calendar, to obtain either earlier or later varieties. As well, both TA and SSC are important considerations when selecting eating apples, as many consumers determine apple quality based on the balance of sweetness and acidity. All the accessions analysed are suitable for direct consumption, since they contain low levels of acidity, high sugar ratios and a wide variation in collection times. In addition, combinations of these different accessions could be used in the processing industry; for example, for producing cider, jams or juices.

The results obtained in this work reveal the high potential of local cultivars. The analysis of compounds carried out within this genetic diversity has revealed that there is a striking variability of analyzed variables (20 in total) especially in terms of acid, pH and SSC in genetically different local accessions. Likewise, this collection has shown a higher TA and SSC content, a lower sucrose content and lower sucrose-glucose relationship in comparison to other accessions (Begic-Akagi et al., 2014; Aprea et al., 2017). This study shows the importance of appreciating and conserving local varieties that may represent a genetic reservoir, which could be introduced into improvement programmes for new hybrids. A more detailed knowledge of the variability of morphological and fruit quality traits of these local apple cultivars will be of benefit in the future selection of apple genotypes with improved nutritional quality and suitable processing characteristics for the manufacturing of apple products. This study opens the door to genetic studies of specific food value characteristics, such as bioactive properties, sugar/acid balance, as well as to their suitability for processing industries that could be useful for breeding programs.

Acknowledgments

This research was supported by the grants RTA2015-00052-C02-00 from the Institute for Agriculture and Food Research and Technology (INIA) and Group A12 from Aragon Government (Spain).

Conflict of interest

No potential conflict of interest was reported by the authors.

References

Aprea, E., Charles, M., Endrizzi, I., Corollaro, L.M., Betta, E., Biasioli, F., Gasperi, F., 2017: Sweet taste in apple: The role of sorbitol, individual sugars, organic acids and volatile compounds. Sci. Rep. 7, 1-10. DOI: 10.1038/srep44950

Begic-Akagi, A., Spaho, N., Gasi, F., Drkenda, P., Vranac, A., Meland, M., 2014: Sugar and organic acid profiles of the traditional and international apple cultivars for processing. J. Hyg. Engin. Design 7, 190-196.

Blanco, D., Gutierrez, M.D., Mangas, J.J., Noval, A., 1988: Determination of sugars and alcohols in apple juice and cider by HLPLC. Chromatographia 25(8), 701-706

Celik, F., Gundogdu, M., Ercisli, S., Kaki, B., Berk, S., Ilhan, G., Sagbas, H.I., 2018: Variation in organic acid, sugar and phenolic compounds in fruits of historical apple cultivars. Not. Bot. Horti. Agrobo. 2018, 46(2), 622-629. DOI: 10.15835/nbha46211160

Colaric, M., Stampar, F., Hudina, M., 2007: Content levels of various fruit metabolites in the 'Conference' pear response to branch bending. Sci. Hortic. 113(3), 261-266. DOI: 10.1016/j.scienta.2007.03.016

Cornille, A., Gladieux, P., Smulders, M.J.M., Roldán-Ruiz, I., 2012: New insight into the history of domesticated apple: Secondary contribution of the European wild apple to the genome of cultivated varieties. Plos. Genet. 10 May. DOI: 10.1371/journal.pgen.1002703

Chapman, G.W., Horvat, R.J., 1989: Determination of nonvolatile acids and sugars from fruits and sweet potato extracts by capillary GLC and GLC/MS. J. Agric. Food Chem. 37, 947-950.

Doyon, G., Gaudreau, G., St-Gelais, D., Beaulieu, Y., Randall, C.J., 1991: Simultaneous HPLC determination of organic acids, sugars and alcohols. Can. Inst. Sci. Technol. J. 24, 87-94.

Guan, Y., Peace, C., Rudell, D., Verma, S., Evans, K., 2015: QTLs detected for individual sugars and soluble solids content in apple. Mol. Breeding 35(6), 1-13. DOI: 10.1007/s11032-015-0334-1

Harker, F.R., Amos, R.L., Echeverría, G., Gunson, F.A., 2006: Influence of texture on taste: insights gained during studies of hardness, juiciness, and sweetness of apple fruit. J. Food Sci. 71, 77-82.

Hecke, K., Herbinger, K., Veberic, R., Trobec, M., Toplak, H., Stampar, F., 2006: Sugar-, acid- and phenol contents in apple cultivars from organic and integrated fruit cultivation. Eur. J. Clin. Nutr. 60(9), 1136-1140. DOI: 10.1038/sj.ejcn.1602430

Iglesias, I., 2012: La fruticultura como alternativa para la producción de manzana de alta calidad y el desarrollo sostenible de zonas de montaña. Rev. Fruti. 21, 14-31

Iglesias, I., Carbo, J., Bonany, J., 2016: Innovacion Varietal manzana: situación y perspectivas de futuro. Rev. Fruti Especial Manzano 52, 6-37.

Jakobek, L., Barron, A.R., 2016: Ancient apple varieties from Croatia as a source of bioactive polyphenolic compounds. J. Food Compos. Anal. 45, 9-15. DOI: 10.1016/j.jfca.2015.09.007

Keenan, D.F., Valverde, J., Gormley, R., Butler, F., Brunton, N.P., 2012: Selecting apple cultivars for use in ready-to-eat desserts based on multivariate analyses of physico-chemical properties. LWT - Food Sci. Technol. 48(2), 308-315. DOI: 10.1016/j.lwt.2012.04.005

Lanzerstorfer, P., Wruss, J., Huemer, S., Steininger, A., Müller, U., Himmelsbach, M., 2014: Bioanalytical characterization of apple juice from 88 grafted and nongrafted apple varieties grown in upper austria. J. Agric. Food Chem. 62(5), 1047-1056. DOI: 10.1021/jf4051232

Larsen, B., Toldam-Andersen, T.B., Pedersen, C., Ørgaard, M., 2017: Unravelling genetic diversity and cultivar parentage in the Danish apple gene bank collection. Tree Genet. Genomes 13(1). DOI: 10.1007/s11295-016-1087-7

Larsen, B., Migicovsky, Z., Aae Jeppesen, A., Gardner, K.M., Toldam-Andersen, T.B., 2019: Genome-Wide Association Studies in Apple Reveal Loci for Aroma Volatiles, Sugar Composition, and Harvest Date. Plant Genome 12 (2).

Łata, B., 2008: Apple peel antioxidant status in relation to genotype, storage type and time. Sci. Hortic. 117(1), 45-52. DOI: 10.1016/j.scienta.2008.03.011

Mapson, L.W., 1970: Vitamins in fruits. In: Hulme, A.C. (ed.), The biochemistry of fruits and their products, 369-383. Academic Press, London.

Panzella, L., Petriccione, M., Rega, P., Scortichini, M., Napolitano, A., 2013: A reappraisal of traditional apple cultivars from Southern Italy as a rich source of phenols with superior antioxidant activity. Food Chem. 140, 672-679. DOI: 10.1016/j.foodchem.2013.02.121

Persic, M., Mikulic-Petkovsek, M., Slatnar, A., Veberic, R., 2017: Chemical composition of apple fruit, juice and pomace and the correlation between phenolic content, enzymatic activity and browning. LWT - Food Sci. Tech. 82, 23-31. DOI: 10.1016/j.lwt.2017.04.017

Petkovsek, M.M., Stampar, F., Veberic, R., 2007: Parameters of inner quality of the apple scab resistant and susceptible apple cultivars (*Malus domestica* Borkh.). Sci. Hortic. 114(1), 37-44. DOI: 10.1016/j.scienta.2007.05.004

Pina, A., Urrestarazu, J., Errea, P., 2014: Analysis of the genetic diversity of local apple cultivars from mountainous areas from Aragon (Northeastern Spain). Sci. Hortic. 174(1), 1-9. DOI: 10.1016/j.scienta.2014.04.037

Planton, G., 1995. Apple maturity: CTIFL-Eurofru starch code and the starchmeter. The apple starch test as a decision help for harvest. Infos CTIFL.

Queji, M.D., Wosiacki, G., Cordeiro, G.A., Peralta-Zamora, P.G., Nagata, N., 2010: Determination of simple sugars, malic acid and total phenolic compounds in apple pomace by infrared spectroscopy and PLSR. Int. J. Food Sci. Technol. 45, 602-609.

Ramos-Cabrer, A.M., Dìaz-Hernàndez, M.B., Pereira-Lorenzo, S., 2007: Morphology and microsatellites in spanish apple collections. J. Hortic. Sci. Biotech. 82(2), 257-265. DOI: 10.1080/14620316.2007.11512227

Royo Díaz, J.B., Miranda, C., Santesteban, G., Errea, P., 2017: Método armonizado para la caractereización pomológica del manzano (*Malus* × *domestica* Borkh). Monografias INIA 29.

Sato, M.F., Vieira, R.G., Zardo, D.M., Falcao, L.D., Nogueira, A., Wosiacki, G., 2010: Apple pomace from eleven cultivars: an approach to identify sources of bioactive compounds. Acta Sci. Agron. 32, 2935.

Thompson-Witrick, K.A., Goodrich, K.M., Neilson, A.P., Hurley, E.K., Peck, G.M., Stewart, A.C., 2014: Characterization of the polyphenol composition of 20 cultivars of cider, processing, and dessert apples (*Malus* × *domestica* Borkh.) grown in Virginia. J. Agric. Food Chem. 62(41), 10181-10191. DOI: 10.1021/jf503379t

UPOV, 2020: http://www.upov.int/overview/es/upov.html

Van Nocker, S., Berry, G., Najdowski, J., Michelutti, R., Luffman, M., Forsline, P., Alsmairat, N., Beaudry, R., Nair, M.G., Ordidge, M., 2012: Genetic diversity of red-fleshed apples (Malus). Euphytica 185 (2), 281-293. DOI: 10.1007/s10681-011-0579-7

Vanderzande, S., Micheletti, D., Troggio, M., Davey, M.W., Keulemans, J., 2017: Genetic diversity, population structure, and linkage disequilibrium of elite and local apple accessions from Belgium using the IRSC array. Tree Genet. Genomes 13(6). DOI: 10.1007/s11295-017-1206-0

Veberic, R., Trobec, M., Herbinger, K., Hofer, M., Grill, D., Stampar, F., 2005: Phenolic compounds in some apple (*Malus domestica* Borkh) cultivars of organic and integrated production. J. Sci. Food Agric. 85(10), 1687-1694. DOI: 10.1002/jsfa.2113

Vieira, F.G.K., Borges, G.D.S.C., Copetti, C., Amboni, R., 2009: Physicochemical and antioxidant properties of six apple cultivars (*Malus domestica* Borkh) grown in southern Brazil. Sci. Hortic. 122(3), 421-425. DOI: 10.1016/j.scienta.2009.06.012

Waldbauer, K., McKinnon, R., Kopp, B., 2017: Apple Pomace as Potential Source of Natural Active Compounds 2017. Planta Med. 83, 994-1010. DOI: 10.1055/s-0043-111898

Wang, R., McCormick, R., Xuan, H., Streif, J., 2010: Distribution of sugar and organic acid components within the KOB heritage apple cultivar collection. Acta Hortic. 858, 89-98.

Watkins, R., Smith, R.A., 1983. Descriptor List for Apple (Malus). IBPGR/CEC AGPR/IBPGR/82/71, EUR 8354/1. Rome, Italy. https://www.bioversityinternational.org/e-library/publications/detail/apple-descriptors/

Wu, J., Gao, H., Zhao, L., Liao, X., Chen, F., Wang, Z., Hu, X., 2007: Chemical compositional characterization of some apple cultivars. Food Chem. 103(1), 88-93 DOI: 10.1016/j.foodchem.2006.07.030

Zhang, Y., Li, P., Cheng, L., 2010: Developmental changes of carbohydrates, organic acids, amino acids, and phenolic compounds in 'Honeycrisp' apple flesh. Food Chem. 123(4), 1013-1018. DOI: 10.1016/j.foodchem.2010.05.053

ORCID
Lourdes Castel – No orcid
Ana Pina https://orcid.org/0000-0002-4988-9467
Patricia Irisarri https://orcid.org/0000-0003-0773-5133
Pilar Errea https://orcid.org/0000-0002-2889-1728

Address of the corresponding author:
Department of Horticulturae, Agrifood Research and Technology Centre of Aragon (CITA). Instituto Agroalimentario de Aragón – IA2 (CITA-Universidad de Zaragoza). Avda. Montañana, 930, 50059, Zaragoza, Spain
E-mail: perrea@aragon.es